**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| (51) International Patent Classification 5 : | | (11) International Publication Number: |
|---|---|---|
| A61L 15/03, A61K 9/22, 37/36<br>A61K 47/00 | A1 | WO 90/03810 |
| | | (43) International Publication Date: 19 April 1990 (19.04.90) |

(21) International Application Number: PCT/GB89/01184

(22) International Filing Date: 6 October 1989 (06.10.89)

(30) Priority data:
8823649.2 7 October 1988 (07.10.88) GB

(71) Applicants *(for all designated States except US)*: ED GEISTLICH SÖHNE AG FÜR CHEMISCHE INDUSTRIE [CH/CH]; CH-61100 Wolhusen (CH). HOLMES, Michael, John [GB/GB]; Frank B Dehn & Co, Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : GEISTLICH, Peter [CH/CH]; Haus Seldwyla, Kehrsitenstrasse 19, CH-6362 Stansstad (CH).

(74) Agent: FRANK B DEHN & CO.; Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).

(81) Designated States: AT (European patent), BE (European patent), CH, CH (European patent), DE, DE (European patent), FR (European patent), GB, GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
*With international search report.*

(54) Title: DELAYED RELEASE COMPOSITIONS FOR WOUND HEALING

(57) Abstract

The invention provides delayed release compositions for use in wound healing comprising a hydrogel containing one or more gellable proteins, peptides or polysaccharides interspersed with a hydrophilic polymer said hydrogel being swollen with an aqueous solution containing one or more growth factors selected from epidermal growth factor, human fibroblast growth factor, human insulin-like growth factor and platelet derived growth factor.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|---|---|---|---|---|---|
| AT | Austria | ES | Spain | MG | Madagascar |
| AU | Australia | FI | Finland | ML | Mali |
| BB | Barbados | FR | France | MR | Mauritania |
| BE | Belgium | GA | Gabon | MW | Malawi |
| BF | Burkina Fasso | GB | United Kingdom | NL | Netherlands |
| BG | Bulgaria | HU | Hungary | NO | Norway |
| BJ | Benin | IT | Italy | RO | Romania |
| BR | Brazil | JP | Japan | SD | Sudan |
| CA | Canada | KP | Democratic People's Republic of Korea | SE | Sweden |
| CF | Central African Republic | | | SN | Senegal |
| CG | Congo | KR | Republic of Korea | SU | Soviet Union |
| CH | Switzerland | LI | Liechtenstein | TD | Chad |
| CM | Cameroon | LK | Sri Lanka | TG | Togo |
| DE | Germany, Federal Republic of | LU | Luxembourg | US | United States of America |
| DK | Denmark | MC | Monaco | | |

# DELAYED RELEASE COMPOSITIONS FOR WOUND HEALING

This invention relates to wound healing and in particular to novel delayed release compositions for use in wound healing.

A number of growth factors have been found with are able to stimulate growth of new tissues when applied to open wounds. There are problems, however, in applying such factors in the optimal way to ensure continued growth while maintaining the sterility of the wound. We have now found that certain hydrogels more particularly defined below are surprisingly more suitable than other compositions investigated for the application and sustained release of a number of polypeptide growth factors.

The growth factors here concerned include Epidermal Growth Factor, Fibroblast Growth Factor, Insulin-like Growth Factor and Platelet Derived Growth Factor.

In particular, the following Growth Factors are particularly well released by the hydrogels here concerned:

Epidermal Growth Factor - Compound 1

Asn Ser Tyr Pro Gly Cys Pro Ser Ser Tyr
Asp Gly Tyr Cys Leu Asn Gly Gly Val Cys
Met His Ile Glu Ser Leu Asp Ser Tyr Thr
Cys Asn Cys Val Ile Gly Tyr Ser Gly Asp
Arg Cys Gln Thr Arg Asp Leu Arg Trp Trp
Glu Leu Arg.

Human Fibroblast Growth Factor - Compound 2

ProAlaLeuProGluAspGlyGlySerGlyAlaPheProProGlyHisPheLysAsp
ProLysArgLeuTyrCysLysAsnGlyGlyPhePheLeuArgIleHisProAspGlyArg
ValAspGlyValArgGluLysSerAspProHisIleLysLeuGlnLeuGlnAlaGluGlu
ArgGlyValValSerIleLysGlyValCysAlaAsnArgTyrLeuAlaMetLysGluAsp
GlyArgLeuLeuAlaSerLysCysValThrAspGluCysPhePhePheGluArgLeuGlu
SerAsnAsnTyrAsnThrTyrArgSerArgLysTyrThrSerTrpTyrValAlaLeuLys
ArgThrGlyGlnTyrLysLeuGlySerLysThrGlyProGlyGlnLysAlaIleLeuPhe
LeuProMetSerAlaLysSer

Human Insulin-like Growth Factor - Compound 3

Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu
Val Asp Ala Leu Gln Phe Val Cys Gly Asp
Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly
Tyr Gly Ser Ser Ser Arg Arg Ala Pro Gln
Thr Gly Ile Val Asp Glu Cys Cys Phe Arg
Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr
Cys Ala Pro Leu Lys Pro Ala Lys Ser Ala

Platelet Derived Growth Factor Compound 4

```
                          20                  40                  60
A-chain MRTLACLLLLCCCYLAHVLAEEAEIPREVIERLARSQLHSIADLQRLLEIDSVGSEDS LDTSL
B-chain MNRCWPLFLSLCQYLRLVSAEGDPIPEELYEMLSDHSIPSFDDLQRLLHGDPGEEDGAELDLNM

                     80                  100                 120
A-chain RAHGVHATKHVPGKRPLPIRRKRSIEEAVPAVCKTRTVIYEIPRSQVDPTSANFLIWPPCVEVKR
B-chain TRSHSGGELESLARGRRSLGSLTIAEPAMIAECKTRTEVFEISRRLIDRTNANFLVWPPCVEVQR

                 140                 160                 180
A-chain CTGCCNTSSVKQQPSRVHHRSVXVAKVEYVAKKPKLKEVQVPLEEHLECACATTSLNPDYREEDT
B-chain CSGCCNNRNVQQRPTQVQLRPVQVRKIEIVRKKPIFKKATVTLEDHLACKCETVAAARPVTRSPG

                 210
A-chain GRPRESCKKAKAKALKPT
B-chain GSQEQRAKTPQTRVTIRTVAVRRPPKGKHRKFKHTHDKTALKETLGA
```

It will be appreciated that analogues of the above growth factors, for example from different annual species which differ from the above sequences by a few amino acids, will be expected to behave in the same way in the gel formulations of the invention.

The above growth factors may be in the natural form or may be made by recombinant DNA technology. In the latter case up to 20%, e.g. 10 amino acid units, may be varied provided the growth factor activity is retained. Additional amino acids or sequences may be added at the N- and C-terminal ends, e.g. signal sequences or methionine at the N-terminal or amino acids corresponding to stop codons. Salts of the polypeptides are also included.

The above growth factors can be obtained from Amgen Inc. of Thousand Oakes, California.

The aqueous hydrogels which carry the growth factors are the hydrogels of USP 4,556,056, a commercial embodiment of which is sold under the name Geliperm, and related materials.

The hydrogels will normally comprise at least on gellable protein, polypeptide or polysaccharide interspersed with at least one hydrophilic polymer and be swollen with an aqueous solution containing one or more of the said growth factors, optionally together with nutrients and or other growth factors.

The hydrophilic polymer in the hydrogel may for example be a polymer of a hydrophilic acrylic or methacrylic acid derivative or vinylpyrrolidine. The acrylic or methacrylic acid derivative is preferably an amide, as in polyacrylamide which is the preferred polymer, or an ester with an alkanol or polyol. The chains of the polymer will normally be interspersed with the chains of the gellable substance preferably by polymerisation in the presence of a solution

of the latter. Apart from a polymerisation initiator, a crosslinking agent such as N,N$^1$-methylene-bis-acrylamide may be present.

The gellable substance is preferably a polysaccharide, agar-agar being particularly preferred; of the gellable proteins, gelatin is preferred.

The water content of such a hydrogel can be very high, for example in the range of 95 to 98% by weight, preferably about 97%. Thus, the solid matrix of the gel may constitute only 2 to 5% by weight of the gel, preferably about 3%.

In general, the most preferred hydrogels comprise (a) agar-agar together with (b) polyacrylamide cross-linked with about 2% by weight of N,N'-methylene bis-acrylamide, advantageously in the ratio range 1:3 to 1:4, preferably about 1:3.5. This gel, when fully swollen with water, contains about 96.5% by weight of water. A gel of this type is now commercially available from Geistlich Pharma of Wolhusen, Switzerland, under the Registered Trade Mark Geliperm.

The hydrogel will generally take the form of a sheet for use as a dressing for direct application to the wound. Such dressings have the advantage of very good compatibility and ease of removable without damage to the growing tissue. In order to accommodate exudation from the wound, the dressings may advantageously be perforated.

The aqueous medium within the gel may usefully contain the essential amino acids and trace minerals normally provided for wound alimentation.

Hydrogel dressings according to the invention may be used in surgery in the preparation of the wound base for free skin transplantation; in the treatment of the donor site after the removal of split skin grafts in plastic surgery and for covering superficial operation wounds to prevent exposed

bradytrophic tissue (tendons, periostium, bone or cartilate) from drying out. In dermatology, the hydrogel dressings may be used in the treatment of both fresh and chronic damage to the epithelium e.g. after dermal abrasion to encourage granulation and the formation of cellular tissue in chronic ulcers, especially crural ulcers, decubitus sores etc; in the treatment of patients with polyvalent allergies when other forms of dressing and external applications are contra-indicated; and in the treatment of superficial thrombo-phlebitis in combination with external therapeutic measures used in such cases.

The following Example is given by way of illustration only:

Example 1

20 g of agar-agar are suspended under agitation in 880 g of deionized water and heated to 95°C until complete dissolution. 1 litre of a second aqueous solution containing 70 g of acrylamide and 1.84 g of N,N'-methylene-bis-acrylamide is prepared at ambient temperature and added to the first solution with thorough mixing. Under continued agitation, 2.2 g of N,N,N',N'-tetrakis-(2-hydroxypropyl)-ethylene diamine dissolved in 60 g of water and then 1.26 g of ammonium peroxidisulfate dissolved in 40 g of water are added.

The mixture is poured into flat moulds (26 x 12mm) to a depth of 3mm.

The mixture has a temperature between 50°C and 55°C and begins to polymerize immediately. After 10 minutes the gel point is reached. The batch is allowed to cool down overnight during which time polymerization is completed.

The gel is freed from soluble impurities by washing with pure flowing water for 24 hours. With this washing the gel swells to 135% of its original weight. Such sheet material is now commercially available under the name Geliperm from Geistlich Pharma of Wolhusen, Switzerland.

The gel is partially dehydrated as described in USP 4 556 056, Example 6 and immersed in a 5% solution of Epidermal Growth Factor (Compound 1) (Amgen Inc, Thousand Oakes, California) until fully swollen. After packaging in polyethylene, the sheet is sterilised by gamma radiation.

CLAIMS:

1. Delayed release compositions for use in wound healing comprising a hydrogel containing one or more gellable proteins, peptides or polysaccharides interspersed with a hydrophilic polymer said hydrogel being swollen with an aqueous solution containing one or more growth factors selected from epidermal growth factor, human fibroblast growth factor, human insulin-like growth factor and platelet derived growth factor.

2. Compositions as claimed in claim 1 in which the gellable component is agar-agar.

3. Compositions as claimed in claim 1 or claim 2 in which the hydrophilic polymer is polyacrylamide.

4. Compositions as claimed in claim 1 containing 95 to 98% by weight of water.

5. Compositions as claimed in claim 1 in the form of sheets for use as wound dressings.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/01184

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 L 15/03, A 61 K 9/22, 37/36, 47/00

## II. FIELDS SEARCHED

Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| IPC5 | A 61 K; A 61 L |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

| Category [10] | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No. [13] |
|---|---|---|
| P,Y | EP, A2, 0312208 (ETHICON INC.) 14 April 1989, see page 3, lines 36-43; page 4, lines 14-59 | 1-5 |
| Y | EP, A2, 0137743 (ED. GEISTLICH SÖHNE A.G. FÜR CHEMISCHE INDUSTRIE) 17 April 1985, see page 3, paragraph 4; claims | 1-5 |
| Y | DE, A1, 3744289 (POLITECHNIKA LODZKA) 14 July 1985, see the whole document | 1-5 |

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 8th December 1989 | 08. 01. 90 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| EUROPEAN PATENT OFFICE | T.K. WILLIS |

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No |
|---|---|---|
| Y | EP, A2, 0272149 (COLOPLAST A/S) 22 June 1986, see column 6, lines 4-45 -- | 1-5 |
| Y | GB, A, 2146335 (EJ ASSOCIATES INC) 17 April 1985, see page 3, lines 24-28, claims 7 and 12 -- | 1-5 |
| Y | EP, A2, 0267015 (ETHICON INC) 11 May 1988, see the whole document -- | 1-5 |

---

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 89/01184

SA 31524

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 08/11/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP-A2- 0312208 | 14/04/89 | AU-D- 22235/88 | 23/03/89 |
| EP-A2- 0137743 | 17/04/85 | JP-A- 60081131 | 09/05/85 |
| | | AU-D- 32981/84 | 11/09/86 |
| DE-A1- 3744289 | 14/07/85 | GB-A- 2200643 | 10/08/88 |
| EP-A2- 0272149 | 22/06/86 | NONE | |
| GB-A- 2146335 | 17/04/85 | NONE | |
| EP-A2- 0267015 | 11/05/88 | US-A- 4717717 | 05/01/88 |
| | | AU-D- 80641/87 | 12/05/88 |
| | | JP-A- 63152324 | 24/06/88 |

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82